

Study of phytoplankton communities in warm-water fish farming ponds in Mazandaran province and identification of dominant microalgae

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Abstract

The aim of this research is to investigate the communities and diversity of phytoplankton in typical warm-water fish farming ponds in Mazandaran Province. Since phytoplankton are the largest primary producers in the waters and the first link in the food chain that transfers energy in the ecosystem to organisms at higher trophic levels, knowing the type and composition of the plankton population makes it possible to obtain information about the population dynamics and life cycle of fish, in addition to knowing the amount of production. Therefore, understanding these organisms in any water source is of particular importance. To conduct this study, three ponds were selected from the farm, and then three samples were prepared from each pond. 500 cc of pond water was taken from three points of the pond by a rotter, poured into a bottle, and fixed in the same area with 4% formalin to be presented in the laboratory for identification and determination of the abundance and biomass of microalgae. The samples were transferred to the planktonology laboratory of the Caspian Sea Ecology Research Institute and the Inland Waters Aquaculture Research Institute for analysis, and then the samples were identified and their abundance was determined in the laboratory. In this study, a total of 61 species from 5 phytoplankton phyla were observed, of which 15 species belonged to the Bacillariophyta phylum, 21 species to the CHOLOROPHYTA phylum, 14 species to the CYANOPHYTA phylum, 7 species to the EUGLENOPHYTA phylum, and 4 species to the PYROPHYTA phylum. The highest density belonged to the phylum Cyanophyta with 67% of the total phytoplankton population, followed by the phylum Chlorophyta with 20% of the population. In this study, the phylum Bacillariophyta was in third place with 12% of the total density. The Euglenophyta group had a very small share (1%) in these pools, and the phylum Pyrophyta was very insignificant.

Keywords: Phytoplankton, Fish Farming Ponds, Density, Mazandaran

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I. Introduction

Phytoplankton are one of the important links in the food cycle in all aquatic resources. All living organisms directly and indirectly require them for nutrition. Knowledge of the type and composition of the plankton population provides the opportunity to obtain information not only on the amount of production, but also on the dynamics of the population and the life cycle of fish. Therefore, it is of particular importance to identify these organisms in any water source (Sarkar *et al.*, 2023 & Jahan *et al.*, 2023). Also, in the aquaculture industry, fish farming ponds are of great importance in terms of providing natural foods in the bed and water column.

Phytoplankton are the largest primary producers in the waters and the first link in the food chain that transfers energy in the ecosystem to organisms at higher trophic levels and in carp breeding ponds, phytoplankton species are usually fertilized through fertilization. Therefore, knowledge of the species composition and density of phytoplankton communities in ponds is inevitable.

Aquaculture is a global activity that has been effective in improving nutrition and contributing to the economic development of third world countries (Rocha *et al.*, 2022). The relatively limited capabilities of fishing and exploitation of natural water resources have made aquaculture, including fish, inevitable to provide animal protein, especially meat, for human consumption.

Today, warm-water fish have a special place in the country, accounting for more than 70% of farmed aquatic products (Thomas, 1983). In Iran, fish farming began with the import of Chinese and common carp and their cultivation in large farms (Sulehria *et al.*, 2013).

Spirulina platensis is rich in nutrients such as protein, various vitamins and minerals, carbohydrates, gamma-linolenic acid, phycocyanin, chlorophyll, and carotenoids (Agustini et al., 2015). This algae contains 50-70% protein, the amount of which varies depending on the cultivation conditions (Alfadhly et al., 2022).

It also contains a wide range of flavonoid and phenolic compounds, including organic acids such as caffeic, chlorogenic, quimic, salicylic, synaptic, and transcinnamic, which have high natural antioxidant activity and it also has a special place in today's food industry due to its content of essential amino acids, vitamin B12, various pigments (carotenoids, phycocyanins, and chlorophylls), unsaturated fatty acids (omega-3), and elements such as iron, manganese, zinc, potassium, calcium, and phosphorus (Pradhan et al., 2022; Marques et al., 2014). Also, the lack of a cellulose cell wall in this algae makes it easier to absorb nutrients, and the low level of nucleic acid in this part (less than 4%) is another advantage of this microalgae over other microalgae (Pradhan et al., 2022).

II. Materials and Methods

To conduct this study, water sampling from the breeding ponds of three selected farms was carried out in the months of May, June and August using a Rotner sampler. Three ponds were selected from the farm and then three samples from each pond were transferred to the planktonology laboratory of the Caspian Sea Ecology Research Institute for identification and then the samples were identified and their abundance was determined in the laboratory.

Thus, 500 cc of pool water was taken from three points of the pool by a rotner, the sample was poured into a bottle, and fixed in the same area with 4% formalin to be presented to the laboratory for identification and determination of the abundance and biomass of microalgae and immediately after transferring the samples to the glass bottle, a label with the relevant information (station name and sampling time) was attached to each sample based on predetermined codes.

In the laboratory, the samples underwent preparation steps (siphoning and centrifugation), qualitative and quantitative examination, and in the sedimentation stage, the water bottles were kept in a dark and still place for at least two weeks, after which the supernatant water of the samples was siphoned off under the hood (on a stable surface that does not cause water to splash), so that the sample volume was almost halved (250 cc). Then, the samples were then centrifuged for 5 minutes at 3000 rpm to obtain a final volume of 40-50 cc. After initial preparation, the samples were kept in a static place for at least 24 hours for qualitative examination.

In the qualitative study, the surface water (without phytoplankton) was transferred to a glass container and 1-2 drops of the bottom water were qualitatively examined with a 22×22 slide and a binocular microscope. 24 hours after the qualitative study, the samples were quantitatively examined.

In this way, based on the density results in the qualitative study (low, medium, and high), the groundwater sample (containing phytoplankton) was brought to a certain volume and then 0.1 cc was removed from it using a grooved piston pipette and they were examined with a 22×22 slide and a microscope with a magnification of 400×, and finally, according to the dilution factor, the density in cubic meters was calculated (APHA, 2017).

Water sampling was carried out monthly during June, August and October to identify and determine the abundance and biomass of microalgae. In the laboratory, the samples were identified and counted using valid identification keys (Proshkina-Lavrenko and Makarova, 1968; Zabelina et al., 1951; Hartley et al., 1996 and Carmelo, 1997) and then the density was calculated (APHA, 2017).

III. Results

Based on the results, a total of 61 species from 5 phytoplankton phyla were observed, of which 15 species belonged to the Bacillariophyta phylum, 21 species to the CHOLOROPHYTA phylum, 14 species to the CYANOPHYTA phylum, 7 species to the EUGLENOPHYTA phylum, and 4 species to the PYROPHYTA phylum. From the Bacillariophyta phylum, *Cocconeis skvortzii*, *Diatoma* sp, *Nitzschia amphibaba* were the most abundant species, and from the CHOLOROPHYTA phylum, *Crucigenia tetrapedi*, *Oocystis solitaria*, *Sheroderia* sp were dominant. The dominant species of the CYANOPHYTA phylum included *Anabaena spiroides*, *Anabaenaopsis nadsonii*, *Chroococcus* sp., *Merismopedia minima*, *Merismopedia pancuata*, *Oscillatoria* sp, and *Spirulina laxissimas*. The species densities of the EUGLENOPHYTA and PYROPHYTA phyla were not significant.

Table 1. Density of different phytoplankton species (number per cubic meter $\pm$ standard deviation) in the studied ponds

Phylum	species	Pond 1	Pond 2	Pond 3
Bacillariophyta	<i>Cocconeis skvortzii</i>	1224 $\pm$ 790	333 $\pm$ 43	38710 $\pm$ 729
	<i>Complidiscus</i> sp.	375 $\pm$ 392	0 $\pm$ 0	0 $\pm$ 0
	<i>Cyclotella meneghiniana</i>	2200 $\pm$ 28	4367 $\pm$ 160	2166 $\pm$ 132
	<i>Cymbella ventricosa</i>	0 $\pm$ 0	166 $\pm$ 437	166 $\pm$ 437
	<i>Diatoma vulgar</i>	0 $\pm$ 0	250 $\pm$ 34	250 $\pm$ 34
	<i>Gomphonema olivaceum</i>	141 $\pm$ 40	85 $\pm$ 22	83 $\pm$ 22
	<i>Gomphonema cotslatum</i>	0 $\pm$ 0	0 $\pm$ 0	704 $\pm$ 450
	<i>Gyrosigma</i> sp.	0 $\pm$ 0	666 $\pm$ 367	2074 $\pm$ 855
	<i>Navicula cryptocephal</i>	6150 $\pm$ 212	0 $\pm$ 0	0 $\pm$ 0
	<i>Diatoma</i> sp.	290 $\pm$ 31	662 $\pm$ 249	10947 $\pm$ 808
	<i>Nitzschia acicularis</i>	6900 $\pm$ 142	7488 $\pm$ 301	584 $\pm$ 160
	<i>Nitzschia amphiba</i>	1233 $\pm$ 494	0 $\pm$ 0	0 $\pm$ 0
CHOLOROPHYTA	<i>Nitzschia sublinaris</i>	3243 $\pm$ 404	19150 $\pm$ 135	27101 $\pm$ 922
	<i>Skeletonema</i> sp.	0 $\pm$ 0	414 $\pm$ 31	416 $\pm$ 32
	<i>Surirella elegans</i>	0 $\pm$ 0	83 $\pm$ 38	1491 $\pm$ 364
	<i>Ankistrodesmus falcatus</i>	5108 $\pm$ 602	4646 $\pm$ 162	334 $\pm$ 38
	<i>Binuclearia lauterbornii</i>	0 $\pm$ 0	5000 $\pm$ 1540	5000 $\pm$ 1540
	<i>Chlamydomonas</i>	140 $\pm$ 31	1167 $\pm$ 498	1166 $\pm$ 498
	<i>Chlorella</i>	33 $\pm$ 24	2250 $\pm$ 22	5123 $\pm$ 658
	<i>Chlorogonium</i>	0 $\pm$ 0	83 $\pm$ 17	83 $\pm$ 17
	<i>Chodatella</i>	0 $\pm$ 0	333 $\pm$ 31	3713 $\pm$ 1732
	<i>Closteridium</i>	0 $\pm$ 0	0 $\pm$ 0	704 $\pm$ 232
	<i>Coelastrum</i>	0 $\pm$ 0	0 $\pm$ 0	5633 $\pm$ 4945
	<i>Coenococcus</i>	0 $\pm$ 0	0 $\pm$ 0	662 $\pm$ 198

	<i>Coenocystis</i>	0±0	250±33	250±33
	<i>Colestrium sphericum</i>	0±0	333±46	333±43
	<i>Cosmarium granatum</i>	0±0	83±15	787±214
	<i>Crucigenia tetrapedi</i>	6431±5424	14300±5381	8750±3885
	<i>Dictyosphaerium</i>	133±399	750±408	750±408
	<i>Oocystis solitaria</i>	13333±800	19783±3992	6583±3211
	<i>Planktonospheria</i>	0±0	88±21	83±21
	<i>Scenedesmus acuminatus</i>	4078±1084	9900±1389	7824±1229
	<i>Scenedesmus longus</i>	275±647	642±642	0±0
	<i>Scenedesmus quadricauda</i>	2944±6	5398±616	2916±616
	<i>Sheroderia</i> sp.	4991±629	59766±49503	59255±49288
	<i>Tetraedorn minimum</i>	758±758	0±0	0±0
CYANOPHYTA	<i>Anabaena spiroides</i>	1232±101	14633±716	2833±629
	<i>Anabaenaopsis elenkinii</i>	133±147	0±0	0±0
	<i>Anabaenaopsis nadsonii</i>	8112±26	34083±6760	34083±6760
	<i>Aphanothece elabens</i>	415±324	250±19	250±19
	<i>Chroococcus</i> sp.	11712±1578	52048±5004	47916±3821
	<i>Gleocapsa limnetica</i>	0±0	169±227	167±227
	<i>Gleocapsa turgida</i>	0±0	333±177	333±177
	<i>Merismopedia minima</i>	31193±3224	92873±7184	67666±4177
	<i>Merismopedia pancuata</i>	588±447	1755±1924	13481±2064
	<i>Microcystis</i> sp.	2625±1659	1900±939	666±779
	<i>Nostoc</i> sp	177±389	0±0	0±0
	<i>Oscillatoria</i> sp.	38263±4186	190766±20462	397240±18935
	<i>Spirulina</i> sp.	687±359	6250±983	6000±948
	<i>Spirulina laxissima</i>	16488±1561	18283±511	3333±327

EUGLENOPHYTA	<i>Euglena caudate</i>	215±29	219±29	0±0
	<i>Euglena gracilis</i>	388±78	0±0	0±0
	<i>Euglena</i> sp.	55±8	2000±1640	2704±2217
	<i>Euglena</i> sp.1	266±4	0±0	0±0
	<i>Euglena viridis</i>	208±110	0±0	0±0
	<i>Euglena wangi</i>	417±4	0±0	0±0
	<i>Trachelomonas spiculifera</i>	14±1	9333±4083	9333±4083
PYROPHYTA	<i>Glenodinium lenticula</i>	275±275	164±50	167±47
	<i>Goniaulax polyedra</i>	0±0	83±61	83±61
	<i>Peridinium latum</i>	0±0	85±69	82±82
	<i>Rodomonas</i>	0±0	87±8	83±9

The highest density belonged to the phylum Cyanophyta with 67% of the total phytoplankton population, followed by the phylum Chlorophyta with 20% of the population. In this study, the phylum Bacillariophyta was in third place with 12% of the total density and the highest density of *Oscillatoria* sp was 190766±20462 in pond 2 and 397240±18935 in pond 3. Two species of *Spirulina*, *Spirulina* sp and *Spirulina laxissima*, were observed in all three studied ponds, which are used as a food source and nutritional supplement.

The Euglenophyta group had a very small contribution (1%) in these pools, and the Pyrophyta phylum was very insignificant (Figure 1).

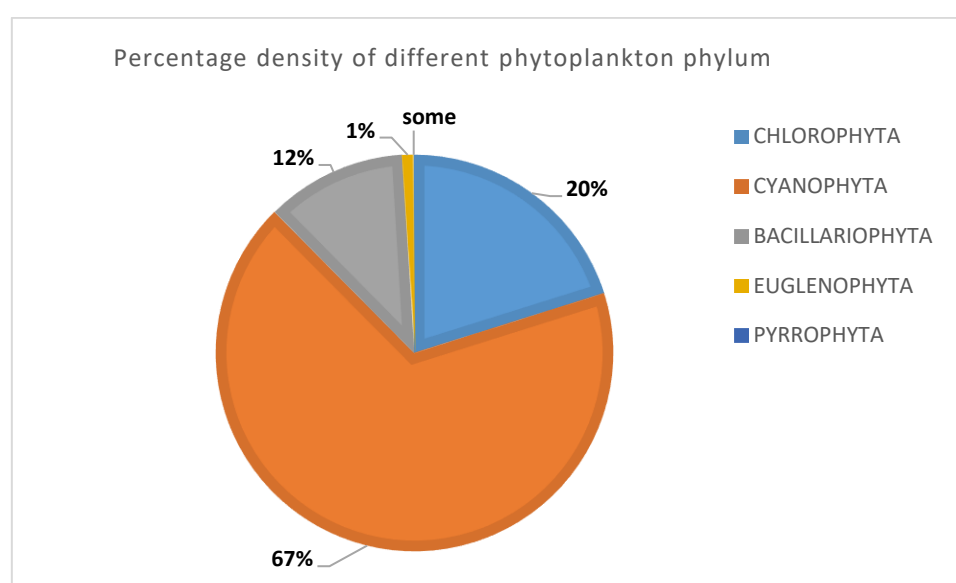


Figure 1. Percentage density of different phytoplankton phylums observed in the studied ponds

IV. Discussion

In this study, a total of 61 species from 5 phytoplankton phyla were observed, of which 15 species belonged to the Bacillariophyta phylum, 21 species to the CHOLOROPHYTA phylum, 14 species to the CYANOPHYTA phylum, 7 species to the EUGLENOPHYTA phylum, and 4 species to the PYROPHYTA phylum were identified. But the highest density belonged to the phylum Cyanophyta with 67% of the total phytoplankton population, and the phylum Chlorophyta was in second place with 20% of the population. In this study, the phylum Bacillariophyta was in third place with 12% of the total density. The group Euglenophyta had a very small share (1%) in these pools, and the phylum Pyrophyta was very insignificant, then, the Cyanophyta phylum was at the top in terms of density, followed by the Chlorophyta phylum, and then the Bacillariophyta phylum. The Pyrophyta phylum always had the lowest abundance. Cyanophyta is one of the algae that is resistant to adverse environments and is able to grow in many unfavorable environmental conditions (Zhigang *et al.*, 2020).

Therefore, it can be concluded that the studied fish farming ponds were in a favorable state in terms of environmental conditions and opportunistic species such as Cyanophyta were able to expand (Ansari-ardali *et al.*, 2022) and the dominance of blue-green and green algae classes in tropical regions (as in the present study) is natural (Xiaodong *et al.*, 2016).

Increased temperature, sunlight, and trophic activities lead to a decrease in water levels, periodic movement of water from depth and nutrient-rich sediments, and increased availability of nutrients for phytoplankton (Ansari-ardali *et al.*, 2022). The breeding ponds of those studies reported that the genera *Anabaena* sp., *Cylindrospermopsis* sp., *Anabaenopsis* sp., and *Aphanizomenon* sp. were the dominant group of cyanobacteria. Also, zooplankton and fragmentation of filamentous species by zooplankton grazing can also increase the density of this phylum (Tahami *et al.*, 2023).

Hassan *et al.* (2002) also identified four groups, Bacillariophyta, Chlorophyta, Cyanophyta, and Euglenophyta, in warm water pools, in accordance with the present study, but the phylum PYROPHYTA was not observed, unlike the present study, and in their study, like this study, the phylum Cyanophyta was the dominant group of microalgae in terms of density.

While Ikpi *et al.* in 2013, investigated the distribution and diversity of phytoplankton in the soil areas of tropical ponds and identified 30 phytoplankton species from 6 phyla that Chlorophyta phylum was at the top with 18 species, but the Euglenophyta phylum was in second place with 5 species, and the Dinophyta and Cryptophyta phylums each had one species.

Also in this study, the Chlorophyta phylum was in second place with 20% of the population, and during the study period, Chlorophyta was the most abundant phylum in all three ponds, and the *Pediastrum simplex* species was the most abundant species in all three ponds and it can be said that the dominance of Chlorophyta in the studied ponds during the warm-water fish breeding season depends on the intensity of sunlight and the increase in phosphate (Celewicz *et al.*, 2022).

The phylum Chlorophyta, or green algae, are mostly freshwater inhabitants that are well fed by fish, and fish farming ponds are most commonly seen in the summer and fall.

The phylum Cyanophyta, or blue-green algae, often prefer warm waters, especially in the summer, and are found in abundance in nutrient-rich waters and the long-term persistence of these algae in fish farming ponds leads to a decrease in water quality, reduced growth, and increased fish mortality.

On the other hand, the physiological and behavioral stability of Chlorophyta causes this class to have better environmental resistance than other species (Celewicz *et al.*, 2022), which is consistent with the results of the present study.

Fish farming ponds, due to their enrichment through chemical and animal fertilizers and high levels of supplemental nutrition, witness an increase in the amount of organic waste and are classified as eutrophic ecosystems.

In terms of economic efficiency and final production amount, the fish farming system in them is carried out in a semi-intensive or intensive manner (Zhong *et al.*, 2011).

In this study, the phylum Bacillariophyta was ranked third with 15 species and 12% of the total density. Members of this phylum constitute a diverse group of freshwater and saltwater algae that play a major role in the nutrition of many zooplankton and aquatic animals, and are indicative of the good biological quality of water for aquaculture in aquaculture ponds.

In the study of Hasan *et al.* in 2002, Bacillariophyceae was in the third place, and in the study of Hegedus *et al.* in 2009, in the study of seasonal and monthly diversity of phytoplankton in fish ponds, this order was in the second place, and it was stated that the presence of this species strongly increases with decreasing temperature gradient, and also with increasing temperature, the orders Chlorophyta and Cyanophyta, followed by Euglenophyta, increase.

One of the factors influencing the high primary production in the studied ponds is the presence of small-sized species, such that small diatoms (*Diatoma* sp.) form a large volume of phytoplankton cells throughout the year in all the studied areas, all of which are factors in increasing primary production and in stations with high concentrations of primary products due to seasonal rainfall, the availability of nutrients and water flow increases

(Tahami, 2017). One of the species in the Bacillariophyta class is *Diatoma* sp. In this study, the species *Diatoma* sp.

It was detected in all 3 ponds. According to (Boyd *et al.*, 1998), the presence of this species in the water indicates the presence of inorganic nitrogen in the water and they attributed the disappearance of this species in some months to low nitrate (3NO) levels and high temperatures.

In the summer, due to the increase in water temperature and the decrease in water level, the concentration of organic matter in the water increases sharply, creating conditions for the growth and reproduction of euglenophyta species. Also, in the study of (Hasan *et al.*, 2002) in warm-water fish ponds, the phylum Euglenophyta was not observed regularly in all samplings and showed low cell density, but in the present study, this phylum was observed in all seasons.

Overall, based on research conducted by Fallahi *et al.*, Bacillariophyta algae have a higher density in the cool months of the year than in the warm seasons and during the period of breeding warm-water fish, due to summer conditions, the growth potential for the cyanobacteria phylum is greater than that of phytoplankton of other phyla (Tahami *et al.*, 2017) and also, the different population structure of algal species plays an important role in primary production in different places and seasons (Tahami & Keyhan-Sani, 2022) and other phytoplankton phylums studied did not have a very high abundance.

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